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### Metabolic engineering for prenylated flavonoids in transgenic plants using bacterial and plant prenyltransferases

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Plants produce a large number of secondary metabolites that do not appear to be relevant for primary biological activity. Among these secondary metabolites, polyphenols are common to all plant species and are known as anti-oxidants and UV protectants. Polyphenols modified with prenyl residues have a variety of biological activities, such as anti-tumor, anti-bacterial, anti-virus, anti-oxidant, anti-tyrosinase and estrogenic. Although prenylated polyphenols are attractive natural products because of their various biological activities, their limited occurrence in plants as well as the difficulty of purification from complicated mixtures has limited the potential applications of these compounds for many years; therefore, metabolic engineering with prenyltransferase genes has been of particular interest. The identification of prenyltransferase genes from *Streptomyces* and *S. flavescens* enabled us to synthesize prenylated polyphenols via metabolic engineering. In this study, *Streptomyces* genes (*NphB*, *SCO7190*, and *NovQ*) and *S. flavescens* genes (*N8DT* and *G6DT*) were used for the targeted production of prenylated polyphenols. We modified the subcellular localization of prenyltransferases to express them in the cytosol, plastids, and mitochondria for soluble-type prenyltransferases from *Streptomyces* (*NphB*, *SCO7190*, and *NovQ*). A total of 624 transgenic *L. japonicus* were generated and analyzed for the production of prenylated polyphenols, and the effect of modification of the subcellular compartmentation of prenyltransferases on the production of prenylated polyphenols was evaluated.

Leaves of transgenic plants were extracted with methanol for chemical analysis using liquid chromatography/mass spectrometry (LC/MS). Unfortunately, none of the transgenic lines accumulated a detectable level of prenylated polyphenol in the leaves. We then supplemented naringenin (100  $\mu$ M) or genistein (100  $\mu$ M) to transgenic leaves and incubated for 24 hours before extraction. 7-*O*-geranylgénistein was detected in transformants with plastid-localized *NphB*, but not detected in transformants with cytosol-localized or mitochondria-localized *NphB*. Both 6-dimethylallylnaringenin and 6-dimethylallylgénistein were detected in plastid-localized *SCO7190* transformants; however, these products were not detected in transformants with cytosol-localized and mitochondria-localized *SCO7190*. Following incubation with 100  $\mu$ M naringenin and genistein, *N8DT* transformants produced 8-dimethylallylnaringenin, the direct enzyme reaction product, and *G6DT* transformants produced 6-dimethylallylgénistein. The overexpression of prenyltransferases in plants yielded only low levels of prenylated polyphenols even after supplementation of the substrate. For *in vivo* prenylation, plastids are the most suitable subcellular compartment for prenyltransferases, both for soluble and membrane bound types. At the same time the problems to be solved are also pointed out, e.g. higher supply of both substrates (i.e. DMAPP and polyphenols). One new strategy will be plastid transformation for the higher production of prenylated polyphenols.

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#### References

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